PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

B4

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 5/00, 15/00, C07H 21/04 | A1

(11) International Publication Number: WO 99/02652

(43) International Publication Date: 21 January 1999 (21.01.99)

(21) International Application Number: PCT/US98/14376

(22) International Filing Date: 10 July 1998 (10.07.98)

(30) Priority Data:
60/052,324 11 July 1997 (11.07.97) US

(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors: HART, Anne, C.; 14 Shipway Place, Boston, MA 02129 (US). FABER, Peter; Apartment 26, 8 Baron Park Lane, Burlington, MA 01803 (US). MACDONALD, Marcy; 462 Waltham Street, Lexington, MA 02173 (US).

(74) Agent: ELBING, Karen, L.; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110–2214 (US).

(81) Designated States: CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: TRANSGENIC NEMATODE MODEL OF TRIPLET REPEAT NEUROLOGICAL DISEASES

(57) Abstract

Disclosed are transgenic nematodes that provide model systems for Triplet Repeat Neurological Diseases (TRENDS).

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

TRANSGENIC NEMATODE MODEL OF TRIPLET REPEAT NEUROLOGICAL DISEASES

Background of the Invention

This invention relates to transgenic animal models of triplet repeat neurological diseases such as Huntington's disease.

Transgenic animal models have recently become valued tools in the elucidation of human disease processes as well as in the characterization of therapeutic drugs for disease treatment. In the case of Huntington's disease, a dominant inherited, untreatable neurodegenerative disorder, a particular need for accurate animal models exists.

The most characteristic feature of Huntington's disease is its peculiar movement disorder which begins subtly and progresses to exaggerated dance-like motions that eventually consume the entire body. Huntington's disease occurs equally in both sexes and is found in all races, but is diagnosed most frequently (i.e., about 1 in 10,000) in people of Western European descent. Although symptoms may begin at any age, they generally appear initially between the ages of 30 and 55, and they progressively worsen until death 12-18 years later.

The clinical progression of Huntington's disease is paralleled by neuronal degeneration in the brain. The hallmark of Huntington's disease is the loss of medium spiny GABAergic projection neurons in a gradient progressing along posteroanterior, dorsoventral, and mediolateral axes of the caudate nucleus. Prior to cell death, signs of neuronal dysfunction are evident in recurved dendritic endings and changes in spine density, shape, and size. The disorder eventually destroys the architecture of the caudate nucleus and the adjacent putamen, although extensive cell loss also occurs in other regions of the basal ganglia and in the deep layers of the cerebral cortex. Overall brain weight may be reduced by 25% or more.

Although the proximate cause of the neuronal dysfunction and death is not yet known, it is ultimately due to the presence of a mutant gene located near the telomere of the chromosome 4 short arm. The Huntington's disease mutation, discovered in 1993, occurs in the first exon of a 67-exon gene encoding a large novel protein. All Huntington's disease

patients have an expansion in a sequence of consecutive CAG codons that lengthens the stretch from the 10 to 34 repeat units seen on normal chromosomes to more than 36 repeat units. Since discovery of the Huntington's disease gene, it has remained unclear how the expanded CAG repeat causes specific neuronal loss. Delineation of similar trinucleotide expansion mutations in a number of other neurodegenerative disorders, including spinocerebellar ataxia I and II, dentatorubropallidoluysian atrophy, and spinal bulbar muscular atrophy, suggests that a common mechanism of neuronal toxicity mediated by the mutant genes may be the underlying cause of each of these disorders.

As a step toward curing these diseases, alleviating their symptoms, or retarding their progression, it would be desirable to develop a transgenic animal model exhibiting the main debilitating phenotype of the triplet repeat neurological diseases, that is, neuronal cell dysfunction and loss.

Summary of the Invention

In general, the invention features a transgenic nematode that includes a TREND (triplet repeat neurological disease)-associated nucleic acid sequence operably linked to a promotor allowing expression in neurons.

In preferred embodiments, the expression of the TREND-associated nucleic acid expression is limited to all or a part of the nematode's nervous system; the TREND-associated nucleic acid expresses a TREND protein (for example, the human Huntingtin protein); the animal exhibits progressive neuronal dysfunction (for example, loss of the ASH neurons or M4 neuron functions in the nematode); dysfunction is seen in at least one nerve cell; at least 40% of the animals in a given population show nerve cell dysfunction; the nematode further includes a homozygous loss of function mutation in a gene encoding a cysteine protease, more preferably, in a gene encoding a caspase, and, most preferably, in the ced-3 gene; and the nematode is *C. elegans*, *C. vulgaris*, or *C. briggsae*.

In a second aspect, the invention features a transgenic nematode exhibiting time-dependent loss of neurons.

In preferred embodiments of this second aspect, the nematode exhibits no substantial non-neuronal cell dysfunction prior to the onset of neuronal cell dysfunction; the degree of neuronal cell dysfunction progressively worsens with the age of the nematode; the nematode

exhibits a decreased number of neurons (preferably, loss of at least one neuron, more preferably, two neurons relative to the number of neurons present at the L1 stage); the animal is capable of transferring the neuronal cell dysfunction trait to its offspring; the nematode expresses a TREND-associated nucleic acid (for example, a nucleic acid which encodes the Huntingtin protein, and preferably a human Huntingtin protein (for example, at least the N-terminal 17 amino acids of the human Huntingtin protein covalently bonded to at least 119 additional glutamines, more preferably at least 150 glutamines, and most preferably 173 additional glutamines)); the TREND nucleic acid is a fragment of a naturally-occurring TREND gene including at least the nucleic acids encoding the trinucleotide repeats of the naturally-occurring TREND polypeptide; the expression of the TREND-associated nucleic acid is limited to all or a part of the animal's nervous system; the TREND-associated nucleic acid is expressed under the control of the *osm-10* regulatory region or the nucleic acid is expressed under the control of a regulatory region allowing expression in an M4 neuron; the nematode further includes a homozygous loss of function mutation in a gene encoding a cysteine protease, more preferably, in a gene encoding a caspase, and, most preferably, in the ced-3 gene.

In a third aspect, the invention features a method of producing a transgenic nematode that exhibits progressive neuronal cell death, involving

(a) introducing into a hermaphrodite nematode a nucleic acid that includes a TREND-associated nucleic acid in an expressible genetic construct;

(b) allowing eggs from the hermaphrodite to hatch into offspring and develop; and

(c) identifying at least one offspring containing the nucleic acid that includes the TREND-associated nucleic acid under the control of a regulatory region allowing expression in neurons.

In preferred embodiments, the TREND-associated nucleic acid expressed is a Huntingtin nucleic acid (for example, the nucleic acid encoding at least the N-terminal 17 amino acids of the human Huntingtin protein followed by at least 119, more preferably at least 150, and most preferably at least 173, CAG repeats encoding glutamine (i.e., 96 or 150 additional CAG repeats relative to the naturally-occurring 23 CAG repeat stretch which occurs in the non-disease causing wild-type Huntingtin gene beginning at the nucleic acid encoding amino acid 18)); the expressible genetic construct includes a neuron-specific

regulatory region (e.g., the *osm-10* regulatory region or a regulatory region which directs expression in the M4 pharynx neuron); the nucleic acids are present in an array having a marker gene (e.g., *lin-15*) suitable for microinjection into nematodes; the nematode further includes a homozygous loss of function mutation in a gene encoding a cysteine protease, more preferably, in a gene encoding a caspase, and, most preferably, in the ced-3 gene.

In a fourth aspect, the invention features a method for expressing a nucleic acid sequence of interest in a nervous system-specific manner, involving operably linking the nucleic acid sequence of interest to an *osm-10* regulatory region, the nucleic acid sequence of interest being positioned in a region that, in the naturally-occurring *osm-10* gene, includes all or a part of the OSM-*10* protein-coding region and the nucleic acid sequence of interest being expressed under the control of the *osm-10* regulatory region.

In preferred embodiments, the expression occurs in *C. elegans*; the nucleic acid sequence of interest is a TREND-associated nucleic acid (for example, having the coding sequence for a Huntingtin protein, for example, the N-terminal 17 amino acids of human Huntingtin protein followed by at least 119 CAG repeats encoding glutamines, more preferably at least 150 repeats, or most preferably at least 173 repeats); the expression occurs in non-essential neurons; and all or a part of the protein coding region of *osm-10* has been deleted, for example, the C-terminal region has been deleted, more preferably amino acids 18 to 419.

In a fifth aspect, the invention features a transgene including (a) a nucleic acid sequence of interest linked to (b) an *osm-10* regulatory region, the nucleic acid sequence of interest being positioned in a region that, in the naturally-occurring *osm-10* gene, includes all or a part of the OSM-*10* protein coding region and the nucleic acid sequence of interest being expressed under the control of the *osm-10* regulatory region.

In preferred embodiments, the nucleic acid sequence of interest is a TREND-associated nucleic acid (for example, encoding a Huntingtin protein, for example, encoding at least the N-terminal 17 amino acids of human Huntingtin protein followed by at least 119 CAG repeats, more preferably at least 150 repeats, and most preferably at least 173 repeats); and expression occurs in non-essential neurons.

In a sixth aspect, the invention features a method of testing a substance for efficacy in the treatment or prevention of a TREND-associated disease, involving exposing a nematode

of the invention to the substance and determining the extent of the progressive nerve cell dysfunction exhibited by the nematode following substance exposure, a decrease in the progressive nerve cell dysfunction, relative to an unexposed nematode of the invention, indicating a substance useful for the treatment of a TREND-associated disease. Also included in the invention are substances identified by this method.

In preferred embodiments, the nematode expresses a nucleic acid having a sequence encoding a Huntingtin protein (for example, a human Huntingtin disease protein, for example, at least the N-terminal 17 amino acids of the human Huntington protein, followed by at least 119, more preferably at least 150, and most preferably at least 173, glutamine residues; the nematode is *C. elegans*, *C. vulgaris*, or *C. briggsae*; the determining is done by assaying for loss or delay of loss of the ASH neurons; the assaying is done by dye filling (e.g., with the vital dyes such as DiI, Di0, or FITC); the assaying is done by use of a Green Fluorescent Protein (GFP) protein (preferably the GFP is covalently linked to the TREND polypeptide); the assaying is done by assaying for loss of ASH or M4 neuron dysfunction (e.g., by monitoring nose touch response or starvation); and the assaying is done at approximately seven days after hatching of the nematode or at the time when nerve cell dysfunction normally becomes measurable in the nematode.

As used herein, by "transgenic nematode" is meant any nematode which includes a nucleic acid sequence which is inserted by artifice into a cell and becomes a part of the genetic material of at least some of the cells of the nematode that develops from that cell, e.g., an ASH, M4, or CAN neuron or a CAN cell. Such a transgene may be partly or entirely heterologous to the transgenic animal. The transgene may be part of an extrachromosomal array, or may be integrated into the genome of the animal. Although transgenic *C. elegans* nematodes represent a preferred embodiment of the invention, other transgenic nematodes include, without limitation, transgenic *C. briggsae* and *C. vulgaris*. Such transgenic nematodes may be constructed by standard techniques using the description herein and are included in the invention.

By "progressive nerve cell dysfunction" is meant a loss of normal function of at least one neuron after hatching of the nematode, relative to a nematode lacking the transgene but otherwise genetically identical. Preferably, loss of function is measured by loss of dye

filling, as described herein, or loss of GFP otherwise detectable when expressed from a genetic construction in a nematode lacking the TREND nucleic acid sequences, or by failure of the nematode to survive on a lawn of OP50 bacteria.

By "OSM-10 protein" is meant a naturally-occurring polypeptide sequence encoded by a nucleic acid sequence capable of hybridizing to the nucleic acid sequence of Fig. 1 (SEQ ID NO:1) under high stringency conditions and which is normally present in the ASH, ASI, PHA, and PHB neurons of the nematode *C. elegans*. Preferably, the protein is the protein encoded by the *osm*-10 sequence of Genbank accession #U00037.

By "*osm*-10 protein coding region" is meant a nucleic acid sequence capable of encoding an OSM-10 protein. Preferably the sequence is the coding sequence of the *osm-10* gene provided in Fig. 1 (SEQ ID NO:1).

By "*osm-10* regulatory region" is meant a nucleic acid sequence capable of hybridizing to nucleic acids 12845-13737 of Fig. 1 (SEQ ID NO:1) under high stringency conditions and which is capable of directing nervous system-specific gene expression. Preferably, the sequence is the sequence of the *osm-10* promoter shown in Fig. 1 as nucleic acids 12845-13734 or a fragment thereof.

By "TREND-associated disease" is meant a neurological disease which correlates with an expanded trinucleotide repeat in a gene of symptomatic individuals. The repeat may be a CAG repeat. For example, Huntington's disease, spinal bulbar muscular atrophy (SBMA: Fischbeck, K.H. (1995), Proc. Assoc. Am. Physicians 107:228-230; Brooks, B.P. et al. (1995), Trends Neurosci. 18:459-461), dentatorubral-pallidoluysian atrophy (DRPLA: Burke, J.R. et al. (1994), Nat. Genet. 7:521-524; Nagafuchi, S. et al. (1994), Nat. Genet. 6: 14-18); and spinocerebellar ataxias (SCAS, for example, SCA1, SCA2, SCA3, MJD6, and MJD7: Zoghbi, H.Y. et al. (1995), Semin. Cell Biol. 6:29-35; Pulst, S.M. et al. (1996), Nat. Genet. 14:269-276; Sanpei, K. et al. (1996), Direct. Nat. Genet. 14:277-284; Imbert, G. et al. (1996), Nat. Genet. 14: 285-291; Durr, A. et al. (1996), Ann. Neurol. 39:490-499; Cancel, G. et al. (1995), Am. J. Hum. Genet. 57:809-816; Haberhausen, G. et al. (1995), J. Neurol. Sci. 132: 71-75; Kawaguchi, Y. et al. (1994), Nat. Genet. 8:221-228; Higgins, J.J. et al. (1996), Neurology 46:208-213; Matilla, T. et al. (1995), Ann. Neurol. 38:68-72; Schols, L. et al. (1995), Hum. Mol. Genet. 4:1001-1005; Schols, L. et al. (1995), J. Neurol. Neurosurg. Psychiatry 59:449-450; Zhuchenko, O. et al. (1997), Nat. Genet. 15:62-68; Trottier, Y. et al.

(1995), Nature 378:403-406; and Lindblad, K. et al. (1996), Genome Res. 6:965-971) are TREND diseases. Non CAG triplet repeat diseases are also included.

By "TREND-associated disease phenotype" is meant an increase in nerve cell dysfunction relative to a wild-type nematode. Preferably, the neurons are ASH neurons or the M4 neuron.

By "TREND-associated nucleic acid" is meant any nucleic acid sequence, regardless of length or post-translational modification, that has been, or will be, found to cause in whole or in part a TREND-associated disease or symptom. Included are human TREND genes, functional homologous from other species, and wild-type homologues modified with a sufficient number of trinucleotide repeats, as provided herein. Such nucleic acids include, without limitation, any naturally occurring TREND nucleic acid sequence having trinucleotide repeats of nucleic acid residues relative to the wild-type (non-disease causing) homology. Preferably at least one triplet repeat associated with the appearance of the TREND disease, relative to the non-disease causing homology, is added, more preferably at least 150, even more preferably at least 200, and most preferably more than 300. The term includes nucleic acids which are fragments or precursors of the naturally occurring disease causing gene. Preferably, at least 500 nucleic acids flanking the trinucleotide repeat are present in the TREND-associated nucleic acid. The nucleic acid may be from a CAG or non-CAG triplet repeat TREND gene.

A "TREND-associated protein" is an amino acid sequence encoded by a TREND-associated nucleic acid.

By "huntingtin protein" is meant a protein encoded by nucleic acid capable of hybridizing at high stringency to nucleic acids 316-366 of Fig. 2 (SEQ ID NO:2) and which further includes at least 119, and more preferably at least 173, glutamine residues. Preferably the first 17 amino acids of the polypeptide are the first 17 amino acids of the protein sequence provided in Fig. 3 (SEQ ID NO:3), and the glutamine residues are covalently linked to the C-terminus of the first 17 amino acids.

A "Huntingtin" gene is a nucleic acid sequence which is capable of hybridizing at high stringency conditions to nucleic acids 316-366 of Fig. 2 and which confers a nerve cell dysfunction phenotype when expressed in nerve cells (particularly the ASH neurons).

By "homology" is meant a gene or protein having at least 60%, and more preferably 80%, identity to the human disease-causing TREND nucleic acid over a stretch of at least 200 nucleic acids and which confers TREND-associated nerve cell dysfunction when expressed in

a nerve cell (particularly in an ASH neuron when expressed under the control of the *osm*-10 regulatory region).

By "high stringency conditions" is meant hybridization in 2X SSC at 40°C with a DNA probe length of at least 40 nucleotides. For other definitions of high stringency conditions, see Ausubel, F. et al., 1994, Current Protocols in Molecular Biology, John Wiley & Sons, New York, 6.3.1-6.3.6, hereby incorporated by reference.

By "nerve cell-specific" or "neuron-specific" is meant that expression of a nucleic acid sequence occurs substantially in a nervous system tissue (for example, the ASH, ASI, PHA, and PHB neurons or the M4 neuron), and does not substantially occur in other cells of the nematode. Preferably, the expression of the nucleic acid sequence in the nervous system tissue represents at least a 5-fold, more preferably, a 10-fold, and, most preferably, a 100-fold increase over expression in non-nervous system tissue.

By "regulatory region" is meant a sequence which is minimally necessary for directing transcription and, if appropriate, translation of an associated nucleic acid coding sequence. The term may also include auxiliary sequences that mediate gene expression in response to an external or internal stimulus, for example, expression that is inducible (for example, by temperature or a chemical stimulus) or expression that is tissue-specific (for example, nervous system-specific) or developmental stage-specific. "Regulatory region" sequences are generally located 5' (or "upstream") of the nucleic acid sequence encoding polypeptide, but may be located within or 3' (or "downstream") of the coding sequence.

By "introducing into a hermaphrodite" is meant to encompass any method by which a transgene may be introduced into a nematode's offspring including, without limitation, microinjection of the parent hermaphrodite with nucleic acids.

By "treatment of a TREND-associated disease" is meant the ability to reduce, prevent, or retard the onset of any symptom associated with a TREND disease, particularly those resulting in progressive neuronal cell death.

By "operably linked" or "in an expressive genetic construct" is meant that a nucleic acid sequence and a regulatory sequence(s) are connected in such a way as to permit expression of that nucleic acid sequence, e.g., *osm*-10, *egl*-1, or *arrestin*-1.

By "covalently bonded" is meant joined either directly through a covalent bond or indirectly through another covalently bonded sequence.

As described herein, the current invention provides a number of advantages. First, because transgenic animals are generally useful for the investigation of specific biological processes and for reproducing particular aspects of human disease, the transgenic animals of the invention provide an important and accurate means for screening drugs to isolate therapeutic agents. In particular, the transgenic animals developing progressive nerve cell dysfunction described for the first time herein have the advantage of mimicking the progressive nerve cell dysfunction observed in patients with TREND diseases. In addition, the progress of this critical symptom, *i.e.*, the loss of specific neuronal cells, is easily observable in the same animal or a population of animals hatched together over time. In addition, because these neuronal cell deaths are observable in the nematodes of the invention at least as early as 7 days after egg hatching and are progressive with time, this animal model is also useful for the testing of palliative therapies which delay the appearance of nerve cell dysfunction. Importantly, because this invention provides a transgenic nematode model of TREND diseases with measurable nerve cell dysfunction, compounds may be screened to identify those which alleviate this loss, even absent knowledge of the symptom's underlying biological cause.

In addition, although not strictly required for drug screening, the associated neuropathological symptoms exhibited by the transgenic animal models described herein provide the unique advantage of allowing the investigation of the etiology of TREND diseases. For example, loss of ASH dye filling in the nematode may be correlated with molecular events associated with the etiology of the disease. In addition, treatments which are shown to diminish the occurrence of neuronal cell death may be tested for their ability to selectively improve certain pathological symptoms in higher organisms or patients.

Significant additional advantages of the nematode model are the short generation time, the ability to genetically manipulate large numbers of animals, and the ability to harvest and accurately stage large numbers of animals for high throughput screening.

Another advantage of this invention is the ease with which these transgenic animals are bred to produce identical transgenic offspring. Also, because these transgenic animals may be bred as readily as control animals and because they produce similar members of offspring, the animals of the invention may be generated in sufficient quantity to make them widely and rapidly available to researchers in this field.

With respect to the construct used to generate the particular transgenic animals described herein, the use of the *osm-10* promoter also provides additional advantages. First, this construct is active only in neuronal tissues of the nematode (most particularly the ASH, ASI, PHA, and PHB neurons). In addition, the *osm-10* gene has the advantage of being expressed in non-essential neurons of the nematode. The deletion of a portion of the OSM-10 protein coding region also has the advantage of providing an ideal site for transgene insertion, for example, for the insertion of the nucleic acids encoding the N-terminal 17 amino acids of the Huntingtin gene plus sufficient glutamine residues to produce progressive nerve cell dysfunction.

As described herein, preferred transgenic animals according to the invention include nucleic acid encoding the first 17 amino acids of the human Huntingtin gene plus at least 119 glutamines residues, more preferably at least 173 glutamine residues carboxy-terminal to the 17 N-terminal Huntingtin amino acids.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

Description of the Drawing

Fig. 1 is a schematic drawing showing a portion of the *osm*-10 gene, including the regulatory region.

Fig. 2 is a schematic diagram of the Huntingtin gene.

Fig. 3 is a schematic diagram of the huntingtin protein sequence

Detailed Description

We have developed a model system for the study of Huntington's disease and other triplet repeat-mediated diseases, otherwise known as poly-glutamine or polyQ diseases, in the nematode *C. elegans*. Huntington's disease is an autosomal dominant, neurodegenerative disorder caused by expansion of a poly-glutamine domain in the N-terminus of the huntingtin protein. The mechanism by which the mutant Huntingtin gene causes neurodegeneration is unknown. The length of the poly-glutamine stretch in the huntingtin protein, however, is inversely proportional to the age of onset in humans. We have inserted nucleic acids

encoding the N-terminus of the wild type and several mutant huntingtin proteins into a reporter construct for the *C. elegans osm-10* gene, which is strongly expressed in four classes of neurons. Transgenic worms have been generated, and the survival of neurons in these nematodes has been examined by staining with vital dyes. Our results suggest that only the construct with the longest glutamine stretch causes cell death in one class of neurons (the ASH neurons). No death was observed in young adults (0 to 3 days after hatching), and up to 40% death was seen in old adults (6 to 7 days after hatching), reminiscent of late onset Huntington's in afflicted adults.

The *C. elegans* model permits large scale screening of drugs and chemical therapies for prevention and treatment of Huntington's disease and other triplet repeat neurodegenerative disorders (including spinocerebellar ataxia I and II, dentatorubropallidoluysian atrophy, and spinal bulbar muscular atrophy, as well as non-CAG triplet repeat diseases), and also for other poly-glutamine-mediated diseases in non-neuronal tissues. The *C. elegans* model also permits the identification and analysis of the mechanism of neuronal death mediated by poly-glutamine proteins and the identification of genes and proteins involved in this neuropathology.

Transgenic nematodes

The transgenic animals of the invention are described in detail below. In general, these animals are produced by first creating a construct that includes a promoter that directs nervous system-specific expression linked to a gene associated with a TREND disease. Vectors useful in *C. elegans* are well known in the art (see e.g., Fire et al., Gene (1990) 95; 189-198). This construct is amplified in bacterial cells, purified, and injected into hermaphrodite worms. Resulting offspring that have incorporated the foreign gene into their cells are identified using a linked marker present on the array. From these founder animals, several distinct animal lines are produced. By crossing with wild-type nematodes or nematodes having other mutations of interest, additional strains may be constructed which facilitate screening.

The transgenic animals described herein exhibit progressive neuronal cell death in the absence of changes in the environment or other parameters relative to controls lacking the TREND construct or having the first 17 amino acids of the huntingtin gene homology from

an (undiseased) individual (i.e., the wild-type gene).

Effects of huntingtin mutants on nematode ASH neurons and behavior

The mutant huntingtin proteins expressed in the transgenic nematodes included the first 17 amino acids of the huntingtin protein linked to a 150 (Q150), 95 (Q95), 23 (Q23), or 2 (Q2) glutamine residue carboxy tail. Our results demonstrated that expression of Q150 caused dye filling defects in the ASH class of neurons. At 3 days of age (3d, young adults), 1% of nematodes demonstrated the defect, which increased to 13% at 8 days (8d, senescent). Given the inherent mosaicism of the transgenic lines, these results slightly underestimated the Q150 effect.

Dye filling requires an intact sensory cilium exposed to the environment. Thus, to determine whether the defective dye filling resulted from ASH death or a defect in ASH sensory cilium, neuronal viability was assessed using anti-endogenous OSM antiserum to test for OSM expression. In both Q150-expressing and control animals, essentially all ASH neurons (98%) stained for OSM, and, therefore, were viable. Accordingly, the Q150-mediated dye filling defect was caused by degeneration or truncation of the ASH sensory cilium, rather than cell death. No dye filling defects were observed for the Q95, Q23, or Q2 nematodes at any age.

Co-expression of huntingtin fragments with OSM-10::GFP allowed rapid discrimination between degeneration and cell death, avoiding the problem of underscoring the huntingtin fragment-mediated defects because of mosaicism. Expression of OSM-10::GFP sensitized neurons to the effects of the huntingtin fragments, resulting in cell death as well as an increased rate of dye defects as compared to non-sensitized neurons. In ASH neurons that failed to fill with dye, those that expressed OSM-10::GFP were scored as dye filling defective, whereas those that failed to express OSM-10::GFP were scored as dead. In sensitized neurons, Q150 co-expression resulted in a dye filling defect in 3% of neurons at 3d, which increased to 26% at 8d. Expression of Q150 also caused cell death in 2% of 3d ASH neurons, increasing to 10% at 8d. This death rate was supported by OSM-10 antiserum results using rigorous scoring parameters, which demonstrated that at least 6% of the Q150 expressing ASH neurons were dead.

Expression of Q95 in nematodes with sensitized AHS neurons resulted in dye filling

defects in 7% of the neurons at 8d. At 3d, however, no dye filling defects or cell death were observed. Similarly, both Q23 and Q2 expression had no effect on dye filling defects or cell death in sensitized neurons at any age tested.

Co-expression of the huntingtin fragments with OSM-10::GFP allowed unequivocal identification of the ASH in fixed nematodes. The huntingtin fragments were visualized using the huntingin antiserum HF1 at 3d and 8d. Expression of huntingtin fragments also resulted in cytoplasmic aggregation. In nematodes co-expressing huntingtin fragments as well as OSM-10::GFP, the huntingtin fragments were visualized throughout the sensitized ASH cytoplasm, including processes and axons. At 3d, the Q2, Q95, and Q150-expressing neurons showed diffuse staining of fragments. Furthermore, the Q150 fragments were stained in 20% of the 3d ASH neurons. At 8d, Q95-expressing neurons demonstrated low level aggregation, and 90% of Q150-expressing neurons demonstrated large protein aggregates. The 8d Q2-expressing neurons demonstrated unchanged subcellular localization. The huntingtin fragments were visible using Nomarski illumination and visible light.

The effects of huntingtin fragment expression were also assessed using the ASH-mediated response to nose touch as a behavioral assay. In 3d nematodes expressing either Q95 or Q150 in combination with OSM-10::GFP, the nose touch response was reduced to 64% and 41% of control, respectively. For comparison, animals lacking ASH neurons exhibited a nose touch response reduced to 35% of control. No behavioral effect was observed in nematodes expressing either Q23 or Q2 in combination with OSM-10::GFP, Q150 alone, or OSM-10::GFP alone. Therefore, the expression patterns that caused behavioral defects corresponded to those that produced defects in ASH neurons.

Effects of *ced-3* mutations

The gene *ced-3* encodes a caspase, a cysteine protease from the ICE family. We assessed cell death in sensitized ASH neurons expressing Q2, Q23, Q95, and Q150 in combination with an OSM-10::GFP fusion protein. In nematodes with a functional mutation in the *ced-3* gene and Q150 expression from extrachromosomal arrays, rtEx[7B,C,D], Q150 expression resulted in a 7% dye filling defect, a 4-fold decrease when compared to the 27% defect exhibited in Q150-expressing nematodes with the wild type *ced-3*. Given the apparent role of the caspase in the Q150-mediated dye defect, these results indicated that Q150

activated the apoptotic cell death pathway.

Low level expression of OSM-10::GFP sensitized the ASH neurons. High level OSM-10::GFP expression resulted in dye filling defects in 1% of neurons at 3d and 26% at 8d. The OSM-10::GFP dye filling defects were relatively independent of *ced-3* gene product function. Loss of function *ced-3* mutations eliminated only 24% of the OSM-10::GFP-mediated defect (54% in wild-type animals compared to 41% in *ced-3* mutant nematodes). The dye filling defect virtually disappeared at lower levels of expression with 1% of neurons exhibiting the defect in 8d wild-type nematodes.

To address the contribution of apoptosis to Q150-mediated death, the requirement for *ced-3* function was assessed. Expression of an extrachromosomal array, rtEx53A, caused cell death in 10% of wild-type *ced-3* nematodes, yet caused no cell death in the *ced-3* mutants. To confirm rtEx53A integrity, the extrachromosomal array was crossed back in a wild-type background. This restored the rate of cell death to that observed originally in the wild-type nematodes.

Furthermore, Q150 protein aggregation also required *ced-3* function, at least in part. *Ced-3* wild-type nematodes expressing the rtEX53A array formed aggregates in 86% of neurons. In contrast, only 50% of neurons formed protein aggregates in *ced-3* mutant nematodes. Following return to a wild-type background, protein aggregation was restored to 77% of Q150-expressing neurons.

Apoptosis and polyQ-mediated disease

Using our *C. elegans* model, the involvement of apoptotic cell death in polyQ-mediated disease was directly addressed. CED-3 was the primary effector of apoptosis in *C. elegans*. In *ced-3* mutant animals, the ability of Q150 to cause an ASH dye filling defect was significantly reduced. In sensitized ASH neurons, cell death was completely dependent on *ced-3* function. The role of apoptosis in cell death has not been reported for mouse models.

Protein aggregation in polyQ-mediated disease

The presence of NI has suggested a common pathogenic mechanism for polyQ-mediated neurodegenerative diseases. However, the role of NI per se has not been determined. In C. *elegans*, cellular dysfunction and cell death occurred in the absence of NI,

suggesting that NI played no active role in the disease process. In support of this hypothesis, NI were observed in SCA7 patient brains in regions unaffected by disease and in all expressing tissues in the SCA3/Drosophila model, even those unaffected by SCA3 expanded fragment expression. Rather than observing NI, we observed large and small cytoplasmic aggregates of Q150 and Q95. The wild-type lengths, Q23 and Q2, did not aggregate, indicating that aggregation did not result from ectopic expression of the huntingtin fragment. Non-nuclear polyQ deposits have been observed in dystrophic neurites for HD. Aggregate formation was independent of *ced-3* function, at least in part, given that aggregation was only partially blocked in *ced-3* mutants. This indicated that blocking the disease-causing effects of polyQ fragments still led to significant protein deposits.

Our model provides additional evidence for a mechanism whereby polyQ fragments initiate degeneration and cell death. Although the model does not address the formation of polyQ fragments, it allows the use of the powerful C. elegans genetics to identify genes/pathways that prevent the deleterious effect of polyQ in the (sensitized) ASH.

Construction of the *osm*-10/Huntington's Disease Plasmid

The *osm*-10 promoter/Huntington's disease construct used to generate animals according to the invention was produced as follows.

The *osm*-10 gene was cloned as described below. Constructs were then generated which included nucleotides 12845 to the BamH1 site at nucleotides 13894-13899 of Fig. 1 (SEQ ID NO:1) (from CT20H4). This fragment includes the *osm*-10 promoter region and a sequence that encodes 17 amino acids from the N-terminus of the OSM-10 protein. Huntingtin-encoding nucleic acids were inserted 3' of the OSM-10 nucleic acid sequences. These constructs were as follows: (i) the control construct (HD+) having nucleic acids (Fig. 2, SEQ ID NO:2) encoding amino acids 1-17 of the non-disease causing (wild-type) human Huntingtin protein (Fig. 3, SEQ ID NO: 3); and (ii) the test constructs having an additional 23 (HD23), 95 (HD95), or 150 (HD150) CAG repeats inserted into the polyglutamine-encoding stretch (as amino acids 18-40) of the huntingtin sequence present in the HD+ construct. These nucleic acid sequences were cloned into the vector described in Fire et al. (Gene (1990) 95: 189-198) and used to microinject hermaphrodite nematodes. Transgenic offspring were then isolated and assayed with vital dyes for neuron survival or dysfunction.

This general technique is readily adaptable for use with other TREND genes or other vector systems.

Fluorescein isothiocyanate (FITC), DiO, and DiI starting protocol

A stock dye solution containing 20 mg/ml 5-fluorescein isothiocyanate DiI or DiO in dimethylformamide was stored at -7°C. If kept dry, this stock solution is stable.

For routine genetics, 50 μl of the stock dye solution was mixed with 200 μl of M9 buffer and applied evenly to the surface of a 10-ml NGM plate seeded with a lawn of *Escherichia coli* (Brenner, S. (1974) The genetics of *Caenorhabditis elegans*, Genetics 77:71-94). After a 2 hour incubation period to allow the dye to diffuse into the agar (final concentration 0.1 mg/ml), live animals were transferred to the plate. After staining for 2 hours or, if convenient, as long as overnight, the animals were transferred to an agar plate without dye for at least 10 minutes to remove free dye from the intestine. Once filled, the neurons remained brightly stained for many hours. Stained animals exhibited normal growth rate, brood size, and mating ability.

As an alternative to the plate method of dye staining, worms may instead be soaked in a liquid solution of dye and then placed on plates lacking dye, as with the plate method. This liquid protocol reduced interfering dye fluoresence from the intestine by staining and destaining the animals in ice-cold M9 buffer. Specifically, the chilled animals do not feed and hence no dye enters the intestine. Typically, in this method, animals were washed free of bacteria in M9 buffer alone, stained for 4 hours in cold M9 buffer containing 0.4 mg/ml FITC, washed three times in cold M9 buffer, and then transferred to a seeded agar plate at room temperature.

Mounting for fluorescence microscopy

To carry out fluorescence microscopy, animals were mounted on layers of 5% agar prepared as described by Sulston et al. (Dev. Biol. (1980) 78:542-576). For photography, the animals were anesthetised in 0.5% 1-phenoxy-2-propanol (Koch-Light Laboratories) and mounted on agar containing 0.2% I-phenoxy-2-propanol.

Cloning of the *osm*-10 gene

Sensory neurons can be divided into two groups, neurons like photoreceptor cells that normally detect one type of stimulus and neurons, termed polymodal neurons, that respond to more than one type or mode of stimulation. Laser ablation studies by various researchers demonstrated that the ASH polymodal sensory neurons are primarily responsible for the detection of varied stimuli including nose touch, high osmolarity, and volatile repellents (WBG 10(1):89, CGC 1705, 2314). *C. elegans* can distinguish between nose touch and other stimuli detected by ASH; habituation to nose touch eliminates 60% of the response to nose touch, but leaves intact response to high osmolarity and volatile repellents.

The ASH neurons express both putative mechanoreceptors and chemoreceptors (e.g., DEG-1, R13A1.4, sra-6 and srb-6, (WBG 14(2):90, CGC 1299, 2314)). However, neither null alleles of the deg-1 nor mec mutations perturb responses to stimuli detected by ASH. We have identified mutations that specifically prevent either nose touch response or high osmolarity avoidance. *Osm*-10 has been cloned and characterized, and likely encodes an osmoreceptor. *Osm*-10 (n1602) III animals were found to be severely defective in their response to high osmolarity, yet they responded normally to the other stimuli detected by ASH. This was demonstrated by the fact that 3+/-1% of wild-type N2 animals escaped an 8M glycerol barrier after 10 minutes, while 77+/-5% of n1602 animals escaped.

The *osm*-10 gene was found to map between mec-14 and lin-39 and was identified based on phenotypic rescue. The nucleic acid sequence is shown in Fig. 1. The protein is 419 amino acids long and shows no significant identity to previously identified proteins. The n1602 mutation changes an E to a K at amino acid 199, and fulfills genetic criterion for a null allele. Using *osm*-10::GFP reporter constructs and polyclonal sera, *osm*-10 appeared to be uniformly distributed in the cytoplasm of ASH, ASI, PHA, and PHB sensory neurons. Laser ablation experiments suggest that ASI, PHA, and PHB are not required for osmotic avoidance.

The n1602 mutation causes the substitution of a lysine for a glutamic acid in the putative extracellular domain. Although n1602 acts as a null allele in genetic tests, it may correspond to a partial loss of function. Insertions of the GFP coding sequence in the rescue construct eliminated *osm*-10 rescue activity, but the resulting reporter constructs consistently had GFP expression in four classes of sensory neurons: ASH, ASI, PHA, and PHB. All four

classes of neurons have exposed, ciliated, sensory endings (ASH and ASI in the anterior; PHA and PHB in the posterior).

The predicted expression pattern of *osm*-10 suggests that it is directly involved in osmotic detection.

Screening Systems for Identifying Therapeutics

Based on our experimental results, we have developed a number of screening procedures for identifying therapeutic compounds (e.g., TREND disease preventative and palliative pharmaceuticals) which can be used in human patients. In particular examples, compounds that prevent or delay the loss of nerve function associated with TREND diseases are considered useful in the invention. In general, the screening methods of the invention involve screening any number of compounds for therapeutically active agents by employing any number of *in vivo* experimental systems. Exemplary methods useful for the identification of such compounds are detailed below.

The methods of the invention simplify the evaluation, identification, and development of active agents for the treatment or prevention of TREND-associated conditions, such as nerve dysfunction and nerve cell loss. In general, the screening methods provide a facile means for selecting natural product extracts or compounds of interest from a large population which are further evaluated and condensed to a few active and selective materials. Constituents of this pool are then purified and evaluated in the methods of the invention to determine their activities.

Below we describe screening methods for evaluating the efficacy of a compound as a TREND-preventative or TREND-therapeutic agent. These examples are intended to illustrate, not limit, the scope of the claimed invention.

Test Extracts and Compounds

In general, novel drugs for the treatment of TREND-associated conditions are identified from large libraries of both natural product or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly,

virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their therapeutic activities for TREND disorders should be employed whenever possible.

When a crude extract is found to prevent or delay loss of nerve function, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having TREND disease preventative or palliative activities. The same assays described herein for the detection of activities in mixtures of compounds can be used to purify the active component and to test derivatives thereof. Methods of fractionation and purification of such heterogenous extracts are known in the art. If desired, compounds shown to be useful agents for treatment are chemically modified according to methods known in the art. Compounds identified as being of therapeutic value may be subsequently analyzed

using a mammalian TREND disease model.

There now follow examples of high-throughput systems useful for evaluating the efficacy of a molecule or compound in treating (or preventing) a TREND-associated condition.

Nematode Dye Filling Bioassays

To enable mass screening of large quantities of natural products, extracts, or test compounds in an efficient and systematic fashion, transgenic *C. elegans* having a TREND nucleic acid (e.g., *C. elegans* containing mutations described herein, such as *C. elegans* having the HD150 construct described above) are cultured in wells of a microtiter plate, facilitating the semiautomation of manipulations and full automation of data collection. As discussed herein, compounds that prevent or delay the loss of dye filling of the ASH neurons or prevent starvation due to dysfunction of the M4 neuron are considered useful in the invention. Such compounds are identified by their effect on preventing or delaying the nerve cell dysfunction in *C. elegans* strains carrying those constructs of the invention (as described above).

In particular examples, nematodes bearing the TREND HD150 construct lose the ability to take up dye in the ASH neurons at 7-8 days after hatching. Thus, visual screening or automated screening for the presence or absence of a suitable dye compound at various times after hatching may be used to identify compounds which delay or prevent the loss of dye filling.

If desired, various concentrations of the test compound or extract can be inoculated to assess the dosage effect. Control wells are incubated in the absence of a test compound or extract. Plates are then incubated at 25°C. After the appropriate periods of time, e.g., 2 to 6 days, nematodes from wells are examined for the presence of neuronal dye filling. A delay in loss of dye filling is taken as an indication that the test compound or extract may be effective at inhibiting a TREND-associated condition, and therefore is considered useful in the invention.

Alternatively, a nerve cell dysfunction may arise from expression of a TREND nucleic acid in the M4 neuron. When this occurs the worms starve, unless given special bacteria to feed upon (Avery et al., Neuron (1984) 3:473-485). Accordingly, nematodes

which are exposed to a compound and which survive in the presence of the compound beyond the period of survival for unexposed worms also identify a useful compound.

In addition, *C. elegans* worms expressing TREND nucleic acids may be utilized in assays to obtain additional information on the mode of action of the test compound in the pathogenic TREND disease.

Nose Touch Assay

As an alternative assay, neural function can be assessed using the nose touch assay, as previously described in Kaplan and Horvitz (Proc. Natl. Acad. Sci. (1993) 90: 2227-2231). Briefly, nematodes are placed on an agar surface and an object of appropriate thickness, e.g., a hair, is placed on the surface such that the nematode moves to contact the foreign object with its nose. While wild-type nematodes respond to this noxious stimuli by immediately backing up, mutant nematodes expressing huntingtin fragments fail to respond or have a reduced response to this stimuli.

If desired, the effect of additional mutations, e.g., *ced-3*, on the huntingtin fragment can be assessed. The assay can be conducted after various concentrations of test compounds or extracts are administered to the nematode. If the compound or extract modulates the behavioral response in huntingtin mutant nematodes, e.g., increases the nose touch response towards the wild-type level, then the compound or extract may be effective in inhibiting a TREND-associated condition.

Other Screening Assays

Other drug screening assays may also be performed using nematode models. If desired, such assays may include the use of reporter gene constructs such as a green fluorescent protein (GFP) sequence under the control of the OSM-10 promoter or another promoter allowing expression in a nerve cell affected by expression of a TREND nucleic acid. In these assays, test compounds are evaluated for effects on reporter gene expression in *C. elegans* strains also expressing a TREND nucleic acid (e.g., the HD150 construct). Expression of the TREND nucleic acid, whether isolated from a diseased individual or modified from the wild-type gene, is accomplished according to standard methods, and, if desired, such nucleic acid may be operatively linked to a gene promoter obtained from *C.*

elegans, such as the *osm*-10 regulatory region.

In one particular example, the invention involves the use of a reporter gene that is expressed under the control of a *C. elegans* nerve-specific promoter. Other equivalent promoter elements may also be used in the invention. The promoter element is cloned upstream of any standard reporter gene, e.g., the luciferase or green fluorescent protein (GFP) reporter genes. The reporter gene construct is introduced into an appropriate nematode host (e.g., *C. elegans*) and an analysis of reporter gene activity is determined in the presence and absence of a candidate compound according to any standard method known in the art. Such reporter gene (and host cell systems) are useful for screening for drugs that prevent or delay TREND-associated nerve cell loss or dysfunction.

Antagonists

As discussed above, useful therapeutic compounds include those which prevent or delay loss of ASH dye filling or the loss of M4 function as measured by starvation. To isolate such compounds, ASH dye filling or viability are measured following the addition of candidate antagonist molecules to a culture medium of nematodes containing the TREND nucleic acids (preferably a staged culture). In this manner, candidate antagonists may be directly administered to nematodes and used to screen for their effects on nerve cells.

Candidate modulators may be purified (or substantially purified) molecules or may be one component of a mixture of compounds (e.g., an extract or supernatant obtained from cells). In a mixed compound assay, the nerve cell loss is tested against progressively smaller subsets of the candidate compound pool (e.g., produced by standard purification techniques, e.g., HPLC or FPLC) until a single compound or minimal compound mixture is demonstrated to prevent or delay the TREND-associated condition.

Candidate antagonists include peptide as well as non-peptide molecules (e.g., peptide or non-peptide molecules found, e.g., in a cell extract, mammalian serum, or growth medium on which mammalian cells have been cultured).

Antagonists found to be effective at the level of delay of nematode nerve cell loss may be confirmed as useful in mammalian models.

If desired, treatment with an antagonist of the invention may be combined with any other TREND disease therapy.

Therapy

Compounds identified using any of the methods disclosed herein, may be administered to patients with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to patients. Although intravenous administration is preferred, any appropriate route of administration may be employed, for example, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Transgenic animals of the invention include those which express a TREND-associated nucleic acid in the nervous system of the animal, thereby producing a model system for the study of TREND diseases and the screening of useful therapeutics. Preferably, the expressed TREND-associated nucleic acid is a Huntingtin nucleic acid (for example, a fragment encoding the first 17 amino acids of the human Huntingtin protein linked at the C-terminus to at least 119, more preferably, at least 173 glutamine residues). Other nucleotides which may be expressed in these animals include, without limitation, all or a portion of the genes for SBMA, DRPLA, and the SCAS, including sufficient triplet repeat residues to confer nerve cell dysfunction. (See Gusella et al., Molecular Medicine (1997) 3:238-242; and Nasir et al., Human Molecular Genetics (1996) 5:1431-1435). Also included are non-CAG repeat TREND nucleic acids.

Other Embodiments

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other

potentially useful parenteral delivery systems for antagonists or agonists of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

In general, specific DNA sequences, which have been shown to be involved in TREND diseases, such as that coding for the first 17 amino acids of the Huntington's disease protein linked by the C-terminus to glutamine residue repeats sufficient to cause progressive neuronal cell death, may be obtained by isolation from genomic sources, by preparation of cDNAs from isolated mRNA templates, by recombinant molecular techniques by direct chemical synthesis, or by some combination of these techniques. Once obtained, the TREND-associated gene may be specifically inserted within the OSM-10 protein coding region (for example, as described herein) of the *osm*-10 gene using the techniques described herein as a means to generate adult transgenic nematodes with progressive neuronal cell death. As described herein, the sequence generally will possess its own termination codon. In addition or alternatively, a polyadenylation site may be added following the coding sequence. The use of the HD150 construct and related constructs and methods described herein provides for neuronal cell specific expression of the transgene in the adult animal and generation of transgenic animals with a neuropathological phenotype resembling a TREND disease.

All publications mentioned herein are hereby incorporated by reference.

Claims

1. A transgenic nematode comprising a triplet repeat neurological disease (TREND)-associated nucleic acid sequence operably linked to a neuron-specific regulatory region.

2. The transgenic nematode of claim 1, said expression of said TREND-associated nucleic acid being limited to neuronal cells.

3. The transgenic nematode of claim 1, said TREND-associated nucleic acid encoding a huntingtin protein.

4. The transgenic nematode of claim 3, said huntingtin protein being a human huntingtin protein.

5. The transgenic nematode of claim 4, said TREND-associated nucleic acid encoding a protein comprising the 17 N-terminal amino acids of the human huntingtin protein covalently bonded to at least 119 glutamine residues.

6. The transgenic nematode of claim 5, said TREND-associated nucleic acid encoding a protein comprising the 17 N-terminal amino acids of the human huntingtin protein covalently bonded to at least 150 glutamine residues.

7. The transgenic nematode of claim 6, said TREND-associated nucleic acid encoding a protein comprising the 17 N-terminal amino acids of the human huntingtin protein covalently bonded to at least 173 glutamine residues.

8. The transgenic nematode of claim 1, said nucleic acid sequence being operably linked to an *osm*-10 regulatory region.

9. The transgenic nematode of claim 1, said nematode exhibiting an increase in neuronal cell death.

10. The transgenic nematode of claim 1, said nematode exhibiting progressive neuronal cell death.

11. The transgenic nematode of claim 10, said nematode exhibiting no substantial non-neuronal cell deaths prior to the onset of progressive neuronal cell death.

12. The transgenic nematode of claim 1, said nematode being *C. elegans*.

13. A transgenic nematode, said nematode exhibiting progressive neuronal cell death.

14. The transgenic nematode of claim 13, said nematode exhibiting no substantial non-neuronal cell death prior to the onset of progressive neuronal cell death.

15. The transgenic nematode of claim 13, said nematode being *C. elegans*.

16. The transgenic nematode of claim 13, said nematode exhibiting progressive neuronal cell death of the ASH neurons.

17. The transgenic nematode of claim 13, said nematode transferring said progressive neuronal cell death trait to its offspring.

18. The transgenic nematode of claim 13, said nematode expressing a TREND-associated protein.

19. The transgenic nematode of claim 18, said nematode expressing a huntingtin protein.

20. The transgenic nematode of claim 19, said nematode expressing a human huntingtin protein.

21. The transgenic nematode of claim 20, said nematode expressing a protein comprising the 17 N-terminal amino acids of the human huntingtin protein covalently bonded to at least 119 glutamine residues.

22. The transgenic nematode of claim 21, said nematode expressing a protein comprising the 17 N-terminal amino acids of the human huntingtin protein covalently bonded to at least 150 glutamine residues.

23. The transgenic nematode of claim 18, said TREND-associated protein being expressed under the control of an *osm*-10 regulatory region.

24. The transgenic nematode of claim 1 or 13, said nematode further comprising a homozygous loss of function mutation in a gene encoding a cysteine protease.

25. The transgenic nematode of claim 24, wherein said gene encodes a caspase.

26. The transgenic nematode of claim 25, wherein said gene is *ced-3*.

27. A method of producing a transgenic nematode that exhibits progressive neuronal cell death, said method comprising

(a) introducing into a cell of said nematode a nucleic acid comprising a TREND-associated nucleic acid under the control of a neuron-specific regulatory region;

(b) allowing said nematode containing said cell to reach maturity; and

(c) identifying said nematode containing said nucleic acid comprising said TREND-associated nucleic acid under the control of said neuron-specific regulatory region.

28. The method of claim 27, said TREND-associated nucleic acid encoding a huntingtin protein.

29. The method of claim 28, said TREND-associated nucleic acid encoding a human huntingtin protein.

30. The method of claim 29, said TREND-associated nucleic acid encoding a protein comprising the N-terminal 17 amino acids of the human huntingtin protein covalently bonded to at least 119 glutamine residues.

31. The method of claim 30, said TREND-associated nucleic acid encoding a protein comprising the N-terminal 17 amino acids of the human huntingtin protein covalently bonded to at least 150 glutamine residues.

32. The method of claim 27, said neuron-specific regulatory region being an *osm*-10 regulatory region.

33. The method of claim 27, said nematode being *C. elegans*.

34. The method of claim 27, said nematode further comprising a homozygous loss of function mutation in a gene encoding a cysteine protease.

35. The method of claim 34, wherein said gene encodes a caspase.

36. The method of claim 35, wherein said gene is *ced-3*.

37. A method for expressing a nucleic acid sequence of interest in a neuron-specific manner, said method comprising operably linking said nucleic acid sequence of interest to an *osm*-10 regulatory region, said nucleic acid sequence of interest being positioned in a region that, in the naturally-occurring *osm*-10 gene, comprises all or a part of the OSM-10 protein-coding region, said nucleic acid sequence of interest being expressed under the control of the *osm*-10 regulatory region.

38. The method of claim 37, said expression occurring in a transgenic nematode.

39. The method of claim 38, said nematode being *C. elegans*.

40. The method of claim 37, said nucleic acid sequence of interest being a TREND-associated nucleic acid.

41. The method of claim 40, said TREND-associated nucleic acid being a huntingtin protein.

42. The method of claim 41, said TREND-associated nucleic acid being a human huntingtin protein.

43. The method of claim 42, said TREND-associated nucleic acid encoding a protein comprising the N-terminal 17 amino acids of human huntingtin protein covalently bonded to at least 119 glutamine residues.

44. The method of claim 43, said TREND-associated nucleic acid encoding a protein comprising the N-terminal 17 amino acids of human huntingtin protein covalently bonded to at least 150 glutamine residues.

45. The method of claim 37, said neuron-specific expression occurring in the ASH neurons of a nematode.

46. A transgene comprising (a) a nucleic acid sequence of interest operably linked to (b) an *osm*-10 regulatory region, said nucleic acid sequence of interest being positioned in a region that, in the naturally-occurring *osm*-10 gene, comprises all or a part of the OSM-10 protein-coding region, said nucleic acid sequence of interest being expressed under the control of the *osm*-10 regulatory region.

47. The transgene of claim 46, said nucleic acid sequence of interest being a TREND-associated nucleic acid.

48. The transgene of claim 47, said TREND-associated nucleic acid being a huntingtin protein.

49. The transgene of claim 48, said TREND-associated nucleic acid being a human huntingtin protein.

50. The transgene of claim 49, said TREND-associated nucleic acid encoding a protein comprising the N-terminal 17 amino acids of human huntingtin protein covalently bonded to at least 119 glutamine residues.

51. The transgene of claim 50, said TREND-associated nucleic acid encoding a protein comprising the N-terminal 17 amino acids of human huntingtin protein covalently bonded to at least 150 glutamine residues.

52. A method of testing a substance for efficacy in the treatment of a TREND-associated disease, said method comprising exposing a transgenic nematode of claim 1 or 13 to said substance and assaying for a TREND-associated phenotype exhibited by said nematode following substance exposure, a decrease in said TREND-associated phenotype relative to an untreated control nematode indicating a substance useful for the treatment of said TREND-associated disease.

53. The method of claim 52, said nematode expressing a huntingtin protein.

54. The method of claim 53, said nematode expressing a human huntingtin protein.

55. The method of claim 54, said nematode expressing a protein comprising the N-terminal 17 amino acids of the human huntingtin protein covalently bonded to at least 119 glutamine residues.

56. The method of claim 55, said nematode expressing a protein comprising the N-terminal 17 amino acids of the human huntingtin protein covalently bonded to at least 150 glutamine residues.

57. The method of claim 52, said nematode being *C. elegans*.

58. The method of claim 52, said TREND-associated phenotype being progressive loss of an ASH neuron.

```
12844    ctgcagc ttgacaccga ctggcacaca caaaatatta aactttcagg gtctaatcca
12901 tcacgaaatg aacgaccaca acctcaatgc aaaatgtcat ttatgccaga aatctttcga
12961 gaataagaag acattccgtg aacactgtga attggatcac acaccggaag tggtatgaca
13021 gtattcatta acagattcaa tttgaaatat taattttagg ttagatgtgt attctgcaaa
13081 agctcattcg atcagccact ggaaatgaac gatgaacaat gggcacactt tttcgctcat
13141 atttatgggg ttagtaattc accattcgaa tctagaagtc gattctcctg actgacattt
13201 ttaaaaccat tttttccagg aaatcctcta ctcccgcctt gccgactacg aggatcgtgc
13261 tttgaacact gcatgaatta tatatttgat tcgatggtta gcttaataaa gaatttttct
13321 gttgtacttc aacctttttc tgaaatagga aataatcaca agttgtctga aaaatgtgat
13381 cagctatcca gatcaaacag tgaaaatgtt gtctgcaatg attcatagct gaacatctga
13441 actcttaaat tttcagcgaa tcatgatttg cattgtgata tattattctt atggttgcaa
13501 aagaaaaaag attacgaaaa tatcttgtga cagagtaaga aaccattgaa atttaattaa
13561 tcaccgtgga gacaggtgtg cactctgctc ttattatatt atcaaaacga gaaacgcgtg
13621 agcaagaagt gggcgtgact tggtagaaag gtacttaatc gagatgttga gccaactttc
13681 gagattattc taaaagtttc catatcaata aacttttttg gaaatttcat cttgcaagAT
13741 GTCGAAGGCA ACGTCCGCGT GTTCTCAAAA Ggtgagattt ctgaaataat acttaggatt
13801 aaacttgaaa ttttatccac ataactttat ctaggaaaaa acttttcga tttatgttag
13861 aacctaataa atttcagAAG ACCTCTCTCC TGGATCC                        13897
```

FIGURE 1
SEQ ID NO:1

```
314              ccatggc gaccctggaa aagctgatga aggccttcga gtccctcaag
361 tccttccagc agcagcagca gcagcagcag cagcagcagc agcagcagca gcagcagcag
421 cagcagcagc aacagccgcc accgccgccg ccgccgccgc cgcctcctca gcttcctcag
481 ccgccgccgc aggcacagcc gctgctgcct cagccgcagc cgcccccgcc gccgcccccg
541 ccgccacccg gcccggctgt ggctgaggag ccgctgcacc gaccaaagaa agaactttca
601 gctaccaaga aagaccgtgt gaatcattgt ctgacaatat gtgaaaacat agtggcacag
661 tctgtcagaa attctccaga atttcagaaa cttctgggca tcgctatgga acttttctg
721 ctgtgcagtg atgacgcaga gtcagatgtc aggatggtgg ctgacgaatg cctcaacaaa
781 gttatcaaag ctttgatgga ttctaatctt ccaaggttac agctcgag                828
```

FIGURE 2
SEQ ID NO: 2

```
aa 1   MATLEKLMKAFESLKSFQQQQQQQQQQQQQQQQQQQQQQQPPPPPPPPPPPQLPQPPPQA
aa 61  QPLLPQPQPPPPPPPPPPGPAVAEEPLHRPKKELSATKKDRVNHCLTICENIVAQSVRNS
aa 121 PEFQKLLGIAMELFLLCSDDAESDVRMVADECLNKVIKALMDSNLPRLQLE
```

FIGURE 3
SEQ ID NO:3

SEQUENCE LISTING

<110> The General Hospital Corporation

<120> TRANSGENIC NEMATODE MODEL OF TRIPLET REPEAT NEUROLOGICAL DISEASES

<130> 00786/358WO2

<150> 60/052,324
<151> 1997-07-11

<160> 3

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 1054
<212> DNA
<213> Caenorhabditis elegans

<400> 1

ctgcagcttg acaccgactg gcacacacaa aatattaaac tttcagggtc taatccatca 60
cgaaatgaac gaccacaacc tcaatgcaaa atgtcattta tgccagaaat ctttcgagaa 120
taagaagaca ttccgtgaac actgtgaatt ggatcacaca ccggaagtgg tatgacagta 180
ttcattaaca gattcaattt gaaatattaa ttttaggtta gatgtgtatt ctgcaaaagc 240
tcattcgatc agccactgga aatgaacgat gaacaatggg cacacttttt cgctcatatt 300
tatggggtta gtaattcacc attcgaatct agaagtcgat tctcctgact gacattttta 360
aaaccatttt ttccaggaaa tcctctactc ccgccttgcc gactacgagg atcgtgcttt 420
gaacactgca tgaattatat atttgattcg atggttagct taataaagaa tttttctgtt 480
gtacttcaac ctttttctga aataggaaat aatcacaagt tgtctgaaaa atgtgatcag 540
ctatccagat caaacagtga aaatgttgtc tgcaatgatt catagctgaa catctgaact 600
cttaaatttt cagcgaatca tgatttgcat tgtgatatat tattcttatg gttgcaaaag 660
aaaaaagatt acgaaaatat cttgtgacag agtaagaaac cattgaaatt taattaatca 720
ccgtggagac aggtgtgcac tctgctctta ttatattatc aaaacgagaa acgcgtgagc 780
aagaagtggg cgtgacttgg tagaaaggta cttaatcgag atgttgagcc aactttcgag 840
attattctaa aagtttccat atcaataaac ttttttggaa atttcatctt gcaagatgtc 900
gaaggcaacg tccgcgtgtt ctcaaaaggt gagatttctg aaataatact taggattaaa 960
cttgaaattt tatccacata actttatcta ggaaaaaact ttttcgattt atgttagaac 1020
ctaataaatt tcagaagacc tctctcctgg atcc 1054

<210> 2
<211> 515
<212> DNA
<213> Homo sapiens

<400> 2

ccatggcgac cctggaaaag ctgatgaagg ccttcgagtc cctcaagtcc ttccagcagc 60
agcagcagca gcagcagcag cagcagcagc agcagcagca gcagcagcag cagcagcaac 120
agccgccacc gccgccgccg ccgccgccgc ctcctcagct tcctcagccg ccgccgcagg 180
cacagccgct gctgcctcag ccgcagccgc ccccgccgcc gcccccgccg ccacccggcc 240

cggctgtggc tgaggagccg ctgcaccgac caaagaaaga actttcagct accaagaaag 300
accgtgtgaa tcattgtctg acaatatgtg aaaacatagt ggcacagtct gtcagaaatt 360
ctccagaatt tcagaaactt ctgggcatcg ctatggaact ttttctgctg tgcagtgatg 420
acgcagagtc agatgtcagg atggtggctg acgaatgcct caacaaagtt atcaaagctt 480
tgatggattc taatcttcca aggttacagc tcgag 515

<210> 3
<211> 171
<212> PRT
<213> Homo sapiens

<400> 3

```
Met Ala Thr Leu Glu Lys Leu Met Lys Ala Phe Glu Ser Leu Lys Ser
 1               5                  10                  15
Phe Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
            20                  25                  30
Gln Gln Gln Gln Gln Gln Gln Gln Pro Pro Pro Pro Pro Pro Pro Pro
        35                  40                  45
Pro Pro Pro Gln Leu Pro Gln Pro Pro Pro Gln Ala Gln Pro Leu Leu
    50                  55                  60
Pro Gln Pro Gln Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Gly Pro
65                  70                  75                  80
Ala Val Ala Glu Glu Pro Leu His Arg Pro Lys Lys Glu Leu Ser Ala
                85                  90                  95
Thr Lys Lys Asp Arg Val Asn His Cys Leu Thr Ile Cys Glu Asn Ile
            100                 105                 110
Val Ala Gln Ser Val Arg Asn Ser Pro Glu Phe Gln Lys Leu Leu Gly
        115                 120                 125
Ile Ala Met Glu Leu Phe Leu Leu Cys Ser Asp Asp Ala Glu Ser Asp
    130                 135                 140
Val Arg Met Val Ala Asp Glu Cys Leu Asn Lys Val Ile Lys Ala Leu
145                 150                 155                 160
Met Asp Ser Asn Leu Pro Arg Leu Gln Leu Glu
                165                 170
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14376

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12N 5/00, 15/00; C07H 21/04
US CL :435/455; 800/3, 9, 13, 21; 536/24.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/455; 800/3, 9, 13, 21; 536/24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, EMBASE, MEDLINE, DERWENT BIOTECH ABSTRACTS; CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DESJARDINS et al. 'The Nematode Caenorhabditis elegans as a Model System to Study Neuronal Cell Death.' In: Neuromethods, Vol. 29: Apoptosis Techniques and Protocols. Edited by J. Poirier. Totawa, New Jersey: Humana Press, 1997, pages 255-277, see the entire document.	1-36, 40-45, 47-58
Y	MARGOLIS et al. cDNA cloning of a human homologue of the Caenorhabditis elegans cell fate-determining gene mab-21: expression, chromosomal localization and analysis of a highly polymorphic (CAG)n trinucleotide repeat. Human Molecular Genetics. 1996, Vol. 5, No. 5, pages 607-616, see the entire document.	1-36, 40-45, 47-58

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
14 OCTOBER 1998

Date of mailing of the international search report
23 OCT 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
BRUCE CAMPELL
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14376

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROSEN, A. Huntingtin: new marker along the road to death? Nature Genetics. 13 August 1996, Vol. 13, pages 380-382, see the entire document.	1-36, 40-45, 47-58
Y	GOLDBERG et al. Cleavage of huntingtin by apopain, a proapoptotic cysteine proteiase, is modulated by the polyglutamine tract. Nature Genetics. 13 August 1996, Vol. 13, pages 442-449, see the entire document.	1-36, 40-45, 47-58
Y	BURRIGHT et al. SCA1 Transgenic Mice: A Model for Neurodegeneration Caused by an Expanded CAG Trinucleotide Repeat. Cell. 22 September 1995, Vol. 82, pages 937-948, see the entire document.	1-36, 40-45, 47-58
Y	MANGIARINI et al. Exon 1 of the HD Gene with an Expanded CAG Repeat Is Sufficient to Cause a Progressive Neurological Phenotype in Transgenic Mice. Cell. 01 November 1996, Vol. 87, pages 493-506, see the entire document.	1-36, 40-45, 47-58
P, Y	HART, A.C. Analysis of a Polymodal Sensory Circuit in Caenorhabditis elegans. Journal of Neurochemistry. Abstract D. 20-26 July 1997, Vol. 69 (supplement), page S38, see the entire document.	8, 23, 37-51
Y, P	SMOOR et al. Analysis of C. elegans nematodes transgenic for the start of the HD gene with and without an extended CAG-repeat. American Journal of Human Genetics. Abstract No. 1878. October 1997, Vol. 61, No. 4 (supplement), page A321, see the entire document.	1-36, 40-45, 47-58